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<b>14. ABSTRACT</b> The scalable emergency system is intended to cover the full scale of possible at-sea incidents from the routine to the rare; from the detection and decontamination of a single piece of equipment before it is loaded on a vessel, to the response, rescue, containment and rehabilitation of a vessel in open waters. The system will be able to safely and quickly decontaminate cargo and personnel, as well as entire vessels at sea and in port. This report defines the proposed program plans of the four (4) primary concepts selected for the project.					
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October 17, 2008

**Subject:** Report on Defining Proposed Program, Contract N00014-06-C-0599

Dear Ms. Mangum,

In fulfillment of Contract Line Item No. 0001, Data Item No. A011, *Report on Defining Proposed Program*, of Contract N00014-06-C-0599, *Feasibility and Top Level Design of a Scalable Emergency Response System of Oceangoing Assets*, attached please find a copy of the report.

If you have any questions or comment, please contact me at 484-557-6590 or [edougherty@ablazedevdevelopment.com](mailto:edougherty@ablazedevdevelopment.com).

Very respectfully,

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# Report on Defining Proposed Program

In support of:

## Feasibility and Top Level Design of a Scalable Emergency Response System for Oceangoing Assets

Office of Naval Research Contract No. N00014-06-C-0599

Contract Line Item No. 0001, Data Item No. A011

Submitted to:  
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Submitted by:  
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October 20, 2008

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## Introduction

This report defines the proposed program plans of the four (4) primary concepts selected for the project, *Feasibility and Top Level Design of a Scalable Emergency Response System for Oceangoing Assets*. The proposed programs included in the report and organizations performing the work are as follows:

1. *Scalable Emergency Response System*, Ship Recycling Research Institute.
2. *Chemical Warfare Agent Remediation*, Villanova University.
3. *Microarray Identification of Pathogens*, Villanova University.
4. *Optical Fibers for Detection of Radionuclide Contamination*, Villanova University.



# Chapter 1 - Scalable Emergency Response System (SERS)

Ship Recycling Research Institute

## Introduction

### *Purpose*

*“Maritime security is best achieved by blending public and private maritime security activities on a global scale into an integrated effort that addresses all maritime threats”*

- *The National Strategy for Maritime Security, 2005.*

The Office of Naval Intelligence’s *Worldwide Threats to Shipping Reports*<sup>1</sup> illuminate a critical facet of our strategic environment and point to much needed capability development in the Maritime domain if we are to achieve our National Security Strategy. Extrapolating the second, third and fourth order effects of military and non-military maritime threats in the context of advanced, future technological capability in an increasingly global economy, enables us to understand the criticality of developing and fielding a scalable emergency response system. Nuclear proliferation and proliferation of other weapons of mass destruction, coupled with agile and non-traditional delivery means of those weapons; terrorism; piracy; advanced naval capabilities of nation states; economic warfare and myriad scenarios that could play themselves out in America’s future, all necessitate a cultural mindset shift in our Navy and within our other organizations with maritime capability. Material and non-material solutions providing the right capabilities in our maritime domain to counter those of astute and dynamic adversaries must be executed through unity of effort among our military, government agencies, nongovernmental organizations and first responders. *A Scalable Emergency Response System for Oceangoing Assets* is the first, logical step in providing the necessary material and non-material capability for America’s future strategic environment. The basic premise of the concept is that every component of the response mission, from living quarters to laboratories to decontamination to waste collection, will be packaged in industry standard ocean shipping containers, or TEUs (Twenty-Foot Equivalent Unit), that can be arranged on the deck of the ship – providing rapid, credible, global response options for at-sea incidents affecting America’s national interests. Within the TEU network, a series of corridor modules will provide infrastructure connections. Easy to transport and arrange, the 20-foot long metal shipping containers can be customized to serve any role of the emergency response system. Using a mix of new and existing capabilities, qualified personnel from the military, law enforcement, intelligence, medical, Chemical, Biological, Radiological, and Nuclear (CBRN) response teams and other stakeholder communities will conduct operations and respond to the event, using the assets available in a TEU network.

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<sup>1</sup> [http://www.nga.mil/portal/site/maritime/index.jsp?front\\_door=true](http://www.nga.mil/portal/site/maritime/index.jsp?front_door=true)

The fabrication of the TEUs used on the platform/transport vessel will take place over three major phases: Concept Development, Prototype, and Manufacture-Fielding-Maintenance. The initial concept for each TEU will be combined with any additional research and planning necessary before a physical prototype is constructed. From this prototype, testing and adjustments will be made to result in the final designated boxes, which will then be manufactured in the relatively small required quantities. Finally, post-mission, TEUs should be decontaminated while still on the transport vessel, and then returned to the manufacturing base on an as-needed basis. There, based on the data linked to the RFID tag, any necessary repair may take place, as well as any design changes that are required. Finally, the container can be restocked and returned to its storage facility in preparation for the next decontamination operation.

Personnel at the TEU storage facility will produce blueprints based on the prototypes already created, and from that basis the actual TEUs will be fabricated and/or modified. This pool of engineers to produce blueprints and test the TEU prototypes will be needed for the duration of the design and production parts of this project, and a trained workforce will be needed to produce them as well as maintain TEUs after their return from completed missions.

There will be some specific needs in the manufacturing and maintenance location. Access to various transport methods will likely be required, with the TEUs moving via air, rail and road at different points of their fabrication and use. Experience with maritime issues is necessary, as well as the equipment to manufacture and move the specialized TEUs. A skilled labor force with employees available for engineering, research and development, and manufacturing are also essential to the success of this proposal.

## **Summary**

In addition to the strategic environment and its implications for the maritime domain that we described above, there are two principal drivers for development of a *Scalable Emergency Response System (SERS) for Oceangoing Assets*: 1) strategic guidance articulated in the National Security Presidential Directive 41 and Homeland Security Presidential Directive and 2) findings from a National Research Council Naval Studies Board.

*“It is the policy of the United States to take all necessary and appropriate actions, consistent with U.S. law, treaties and other international agreements to which the United States is a party, and customary international law as determined for the United States by the President, to enhance the security of and protect U.S. interests in the Maritime Domain, including the following:*

- *Preventing terrorist attacks or criminal acts or hostile acts in, or the unlawful exploitation of, the Maritime Domain, and reducing the vulnerability of the Maritime Domain to such acts and exploitation;*
- *Enhancing U.S. national security and homeland security by protecting U.S. population centers, critical infrastructure, borders, harbors, ports, and coastal approaches in the Maritime Domain;*
- *Expediting recovery and response from attacks within the Maritime Domain;*

- *Maximizing awareness of security issues in the Maritime Domain in order to support U.S. forces and improve United States Government actions in response to identified threats;*
- *Enhancing international relationships and promoting the integration of U.S. allies and international and private sector partners into an improved global maritime security framework to advance common security interests in the Maritime Domain; and*
- *Ensuring seamless, coordinated implementation of authorities and responsibilities relating to the security of the Maritime Domain by and among Federal departments and agencies.<sup>2</sup>*

Developing and fielding the SERS for Oceangoing Assets capability directly supports this Presidential directive through its deterrent and/or direct action qualities.

According to the National Research Council, the Navy suffers from an over concentration on preventing contamination at the expense of decontamination (NRC- Naval Studies Board 2004). This project has proposed a modular delivery system based on TEUs to address this gap in naval consequence management. With an additional year of funding the project will deliver functional requirements documents and blueprints of the most critical TEUs for prototype design as well as updates to Tactics, Techniques, and Procedures (TTPs) and training regimes to conform with the new duties and responsibilities this system will require.

Implementation of the TEU SERS concept will require a series of reach-back capabilities that are either missing entirely or that are interspersed across geographic, departmental and agency boundaries as well as the private and educational sectors. The report identifies two actions that should fulfill the need to unify and centralize reach-back capabilities: establish a design, fabrication, and maintenance facility for the modular TEU units that this project will use extensively; and develop a dedicated research and innovation cluster that draws together DOD personnel, university and other researchers, and the private sector.

## **Basic Details**

### ***Description of Capability to be Delivered***

Although the TEUs have gone through a top level conceptual design, major research will still be required to complete the design of some of the containers, specifically those tasked as laboratories. Additional research and planning may be needed to quantify the specific methods to cover and seal the TEUs against potential environmental contaminants while maintaining a hospitable environment within them. Engineering and pharmaceutical/HAZMAT knowledge will be required to complete this portion of the design process.

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<sup>2</sup> National Security Presidential Directive NSPD 41 Homeland Security Presidential Directive HSPD 13, 21 December 2004, pp 2-3

Decontamination equipment will be used to cleanse personnel, sensitive shipboard electronics, general equipment and ship surfaces and internal systems. Isolation chambers will store contaminants and wastewater for safe transport and disposal. Habitability and Advanced Life Support (ALS) modules add additional life supporting space that could also be a part of this complement. Basic Life Support spaces augment organic first aid capabilities. Office modules provide the meeting spaces for DOD, Department of Justice (DOJ), Department of Health and Human Services (DHHS), Department of Homeland Security (DHS), EPA staff, etc., to work together effectively.

TIMELINE ACTION		RESPONSIBILITY
1	Develop concept for each TEU type (laboratory, hospital, wastes/hazmat, office, housing, etc.) designed through research and planning including covering & sealing TEUs against potential environmental contaminants	Research Cluster/TEU Facility
2	Construct physical prototype	TEU Facility
3	Test and make adjustments to prototype	Research Cluster/TEU Facility
4	Design final TEU per TEU type	TEU Facility
5	Manufacture designed TEUs in necessary quantities	TEU Facility
6	Perform maintenance on all TEUs twice yearly in storage	TEU Facility
7	If attack occurs, design specific TEUs based on need	Research Cluster
8	If utilized, decontaminate TEUs while on transport vessel prior to being returned to Port	N/A
9	If utilized, post mission TEUs restocked, repaired and maintenance completed before their return to storage	TEU Facility

## ***Target Acquisition Program***

The components of a full decontamination platform/transport vessel will be modularized in order to maximize the project's flexibility and reach. The modular approach gives the CBRN team the ability to quickly assemble a completely customized response. On the resulting decontamination platform, everything is interconnected and each piece is essential. The platform's footprint and resource use will be kept to a minimum. Another advantage of this model is that it does not require new deliverable technology. It is based entirely on preexisting technology and materials. TEUs are relatively inexpensive and easy to obtain and they require only minor adjustments to make them livable. The end result, therefore, is a completely mission-customizable, interchangeable system of laboratories, living quarters, and other necessities for any possible decontamination operations.

## **Approximate Program Timeline for Specific Capabilities**

- |                    |  |
|--------------------|--|
| <u>Years 1 - 2</u> | Document operational requirements; develop technical blueprint and design documents. |
| <u>Years 3 - 4</u> | Physical prototypes constructed; testing and adjustments to prototypes.              |
| <u>Year 5</u>      | Manufacturing necessary quantities.  |
| <u>Years 6+</u>    | Maintenance, repair and restock.   |

## **Technology Readiness Level (TRL) Estimate**

Based on the information provided in the Technology Readiness Levels Chart in *Appendix A*, the estimated technology readiness is currently at Level 2. Basic principals and paper studies have been observed and developed and the concept has been formed. Practical applications have been considered and introduced. At this stage, research and development of TEUs has not been initiated for this project, however, TEUs have been utilized for similar functional projects. Laboratory studies have not been initiated but development is possible. Critical elements have not yet been identified or demonstrated with innovative users. To reach level three, the TEU concept has to be actively developed in a functional and practical setting.

## ***Strategy to Integrate to a Program***

### **Key participants**

Various engineering capabilities will be required to begin the blueprints and structure of the various TEU designs. There will need to be numerous TEU configurations for each function: housing of personnel, hospitalization, technology center, decontamination, etc.

State and local government financial opportunities have been identified for the Pennsylvania-New Jersey region. State and city economic development departments will be essential for providing loans, grants, and tax incentives for establishing and maintaining engineering firms. These financial supports will be essential for sustaining employment and extension of the project.

The project has conducted extensive research on the development and organization of the TEU design and development. Both Ablaze Development Corp and Ship Recycling Research Institute will be involved in the maturity of the TEU concept, providing research studies, data, and guidance to the preparation and arrangement of the model. NSWC-Center for Innovation in Ship Design, Villanova University-College of Engineering, and Logistics Management Institute have also provided technical and subject matter expertise to the development of the SERS TEU concept.

### **Anticipated contracting partners**

The personnel accompaniment will include various Navy, Marine Corps and Service components as well as civilian personnel from a variety of governmental agencies, including the Department of Homeland Security (DHS), Federal Emergency Management Agency (FEMA), Federal Bureau of Investigation (FBI), Central Intelligence Agency (CIA), Environmental Protection Agency (EPA), Center for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA). These agencies will contribute their particular expertise, research, and unique abilities to the operation in order to accelerate cleanup and increase understanding of the situation. Civilian participation may necessitate initially using the already-existing Navy SOP to collapse personnel into the chain of command.

#### **Agency Partnerships**

1. Environmental Protection Agency
  - a) The EPA's Radiological Emergency Response Team (RERT) specializes in responding to radiation-related incidents.
  - b) Members of the RERT are experienced in using two Mobile Environmental Radiation Laboratories during operations to detect and measure radiation.
2. Centers for Disease Control and Prevention
  - a) Under the National Response Plan, the CDC is designated to protect the health of everyone on site following a radioactive incident.

- b) The CDC will ensure the safety of the NR Module's personnel and provide information on the specific hazards posed by the radioactive contaminants that are identified.
- 3. State Agencies, where appropriate
  - a) When civilian populations are threatened by an incident near shore, officials from state agencies can be reached through the Operations Center.
- 4. Department of Homeland Security
  - a) Under Homeland Security Presidential Directive 7, DHS Secretary is responsible for coordinating protection activities for critical infrastructure sectors for transportation including the maritime domain.
  - b) The DHS Secretary will work closely with other Federal departments and agencies, State and local governments, and the private sector in accomplishing the objectives of HSPD 7.

In addition to these external agency partners, personnel will be contracted to load and unload the TEU modules. The TEUs will be designed to fit together in a structure based on the individualized decontamination response. Employees will also decontaminate and restock the TEU storage facility after a completed mission.

### Expected funding levels

The following table provides an expected funding level required for Year 1 broken down by task. Funding levels for follow on years will be developed during this period.

TASK	BUDGET
Integrate new practices and procedures proposed into existing TTPs as possible and create new TTPs in other cases	\$ 40,000
Rewrite Damage Control men training guidelines to reflect new roles given in the current report	\$ 40,000
Create presentation of phased response system and present to end users	\$ 20,000
Narrow TEU proposals to most useful/important modules	\$ 80,000
Create functional requirements documents for 2-4 TEU modules	\$ 300,000
Research off the shelf component options	\$ 100,000
Resolve Integration issues with and within TEU modules	\$ 150,000
Create blueprints for 1-3 TEU modules	\$ 100,000
Share out blueprints to end users for comment, review and modification	\$ 50,000

Assess ability of Philadelphia Area to create prototypes and possible production of TEU modules	\$ 40,000
Create working group/partnership of local govt. agencies, businesses and universities to advise on blueprints, prototype and production of TEU modules	\$ 60,000
Research incentives and structures to best facilitate research cluster to further work	\$ 20,000
<b>Year 1 Funding</b>	<b>\$ 1,000,000</b>

## Technical and Programmatic Details

### ***Current Status Summary***

The SERS for Oceangoing Assets capability is in conceptual development at the present time. As previously discussed, the estimated TRL rating is between two and three, but no independent analysis of that assessment has been completed. Proof of concept and additional coordination with key stakeholders will inform the appropriate transition into a program within one or more of the National Security stakeholder organizations. Consultation with DHS, as well as other stakeholders, is required. If best suited as a DoD program, then perhaps the Advanced Concept Technology Demonstration (ACTD) affords the most appropriate option for transitioning the concept to a program.

### ***Risk Analysis***

The transport of a TEU delivery vessel may incur complications when accessing the contaminated ship. Effective transportation methods may differ depending on cost, capacity, and speed. As the project continues, the transportation methods can be continually researched and innovative routines attempted.

The usability of TEUs under these extremely hazardous conditions is unknown since they have not been employed in decontamination missions as of yet. Environmental hazards may be presented during decontamination or shipment of the modules. Reuse of TEUs will require additional safety measures at the storage facility.

Beyond the material challenges associated with this concept, there are significant challenges in executing such a capability among several stakeholder organizations - bringing about truly unified action under the most difficult conditions and in extreme environments (at sea, CBRN, etc.). An extensive and comprehensive exercise program, experimenting with various command and control and policy solutions, will be key to mitigating risks associated with implementing, managing and executing this capability.



## ***Capability Development Strategy***

The current capability development strategy is to pursue a DoD solution to a material capability gap in the maritime domain. However, this capability spans potentially all National Security stakeholder organizations and as such will truly test our ability to conceive, develop, test and field a true “unified action” capability. As the National Strategy for Maritime Security puts it, “In many instances each layer of maritime security is the responsibility of a different agency with multiple jurisdictions and functions. Integrating these disparate maritime security layers requires a clear delineation of roles and responsibilities and cannot be achieved through cooperation alone. In particular, to achieve unity of effort and operational effectiveness, maritime security forces from both the U.S. Armed Forces and law enforcement agencies must have the capability and authority to operate in mutually supporting and complementary roles against the spectrum of expected security threats. These security forces must have a high degree of interoperability, reinforced by joint, interagency, international training and exercises to ensure a high rate of readiness, and supported by compatible communications and, where appropriate, common doctrine and equipment.”<sup>3</sup>

The fabrication of the TEUs used on the platform/transport vessel will take place over three major phases. The initial concept for each TEU will be combined with any additional research and planning necessary before a physical prototype is constructed. A comprehensive, multi-Service, multi-agency exercise program will further inform the concept prior to prototype construction. From this prototype, testing and adjustments will be made to result in the final designated boxes, which will then be manufactured in the relatively small required quantities. (Because some designs will need more reproductions than others, e.g., living quarters, corridors, the manufacturing process should be scalable.) Finally, post-mission, the TEUs will need to be restocked, repaired, and have any additional maintenance completed before their return to storage.

Although the TEUs have gone through a top level conceptual design, major research will still be required to complete the design of some of the containers, specifically those tasked as laboratories. Additional research and planning may be needed to quantify the specific methods to cover and seal the TEUs against potential environmental contaminants while maintaining a hospitable environment within them. Engineering and pharmaceutical/HAZMAT knowledge will be required to complete this portion of the design process.

Once the prototypes are completed and approved, the manufacturing phase will begin. A storage facility will be needed, with attached offices for a pool of engineers. These personnel will produce blueprints based on the prototypes already created, and from that basis the actual TEUs will be fabricated and/or modified. Access to various transport methods will likely be required, with the TEUs moving via air, rail and road at different points of their fabrication and use.

This is not envisioned as a large-scale manufacturing project, although the manufacture of even limited numbers of TEUs will create short-term jobs requiring a trained workforce at a

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<sup>3</sup> The National Strategy for Maritime Security, 2005, page 22.

manufacturing base. Multiples of most of the planned TEU prototypes will need to be made, to account for the maximum flexibility of the mission. During manufacture, each TEU should be tagged with a unique Radio Frequency Identification (RFID) tag to aid in later maintenance.

After a completed mission, TEUs should be decontaminated while still on the transport vessel, and then returned to the manufacturing base on an as-needed basis. There, based on the data linked to the RFID tag, any necessary repair may take place, as well as any design changes that are required. Finally, the container can be restocked and returned to its storage facility in preparation for the next decontamination ops.

There will be some specific needs in the manufacturing and maintenance location. Experience with maritime issues is necessary, as well as, of course, the equipment to manufacture and move the specialized TEUs. A pool of engineers to produce blueprints and test the TEU prototypes will be needed for the duration of the design and production parts of this project, and a trained workforce will be needed to produce them as well as maintain TEUs after their return from completed missions.

At this time there have been no activities leading to blueprinting or producing TEUs for the usage of this project. Relevant, local companies that develop and ship TEUs have been identified for possible contact; these companies may be valuable in the design and implementation of the TEU concept. In the future, these or similar companies should be made available for the advancement of the project.

## Chapter 2 – Chemical Warfare Agent Remediation

Villanova University

### Introduction

#### *Purpose*

There is a serious threat to our nation's surface waterways (ports, harbors, rivers, lakes, coastal waters, as well as potable water supplies) from malicious chemical attacks as well as accidents and equipment failures. Except for the use of aerosols, attack to our nations food and water systems would be the most effective means of distributing a chemical or biological agent. Our nation requires a two thronged approach to chemical contamination in water. First an EWS (early warning system) must be developed which can monitor water and provide warning at the onset of contamination. The second requirement is a quick clean-up and decontamination method. This two phase approach of quick remote sensing followed by detailed analysis and treatment has gained growing support as the preferred method for chemical warfare agent monitoring.

In the proposed project we plan to solve the second requirement, by developing a universal method for quick deactivation and removal of chemical pollutants in water. We plan to examine two nanoparticles' ( $\text{TiO}_2$  and  $\text{ZnO}$ ) effectiveness at neutralizing and destroying aqueous solutions of chemicals of interest. We have selected three carbon based chemicals to use as model contaminates for our initial studies:

- m-Dinitrobenzene, a compound released in water during chemical explosive manufacturing and structurally similar to many explosives.
- Dimethyl methylphosphonate, a chemical warfare nerve agent surrogate.
- Thiodiglycol, a building block for many pesticides and similar to chemical warfare agents.

Our preliminary results show that with exposure to sunlight, these nanoparticles can effectively neutralize these contaminates; however, complete oxidation to carbon dioxide, water and mineral salts is not always achieved in less than 24 hours. In other words, after short exposure time, even if all of the starting material has been neutralized, some byproducts do remain which do need to be treated or removed from solution. Therefore, we also propose the use of carbon nanofibers as catalyst supports for the nanoparticles to increase their catalytic activity in hopes of further oxidizing byproducts and unreacted materials. In addition, these fibers will also be used as direct adsorbents to remove chemical contaminants and byproducts from water.

We plan to continue our studies of the model contaminate destruction by exposing aqueous solutions of these chemicals to our  $\text{TiO}_2$  and  $\text{ZnO}$  nanoparticles. These compounds will be studied individually at different concentration levels in both fresh and salt water in the laboratory. We will vary the particle size and amount of each nanoparticle used and also explore the effect of pH, co-solvent, temperature, and UV light exposure on the destruction process. Identification of the byproducts formed will be initiated to help increase our understanding of the destruction

chemical pathway. Carbon nanofibers will also be used as catalyst supports and their effect of the chemical destruction quantified. Finally, the carbon nanofibers will be used as adsorbents themselves. Their ability to remove the initial contaminants as well as byproducts formed will be explored as a function of the processing conditions (temperature, pH, salinity, concentration, UV light, etc.).

## **Summary**

The continuation of this project will provide a set of nanoparticles and/or carbon nanofibers that would be a fast response option for destroying and removing an unknown contaminate from water. There are already a number of commercially available sensors for chemical detection in the water and there have been many publications addressing the area of sensor technology for environmental analysis and monitoring of pollutants, contaminants and chemical hazards. Many different types of sensors have been developed and several types have been found to be adequate for chemical and warfare agent monitoring. Surface acoustic wave sensors, ion mobility spectroscopy, and gas chromatography sensors have had success with the military. Of course more development is underway to improve sensor selectivity, detection limits, robustness in the environment, and to create sensor networks. However, what is not really adequate at this time is the ability to quickly contain and remediate chemical and warfare agents once they have been detected in the water. The Navy needs to be able to dispatch containment and treatment facilities anywhere in the world once chemical or warfare agents have been detected either onboard a ship (such as a Navy vessel or passenger cruise ship), in the water (open, ports, or drinking supplies), or on land (such as in custom areas in ports). The system would have to be able to treat a wide variety of possible chemical contaminants and also be mobile. It would not be economical to have a separate specific treatment process designed for every possible contamination scenario, but rather, one process that could handle any scenario would be ideal. Also, there is not sufficient time to identify the contaminate, find the best treatment process for it, and get that treatment process to the site. During this extensive investigation and analysis, the contaminate would spread throughout the water and become more of a threat to humans and the environment. The system would need to be very reliable, robust, and require minimal man power to operate. In addition to warfare agents, other chemical pollutants which are detected in the water, such as pesticides, explosives, or ordinary organic chemical spills could also be treated. Our system of nanoparticles and/or carbon nanofibers will be designed to be a quickly deployable first response treatment and removal process for any chemical contaminants in water.

Although we are fairly certain our decontamination and removal process will be effective, further studies are required for verification. The Office of Naval Research should be funding this investigation which will take place over the next three years at the cost of \$250,000/year for a total of \$750,000 during the investigation phase. The next phase would be production and/or purchase of the required nanofibers and nanoparticles for implementation on a fast response vessel. Required funding of \$3 million would be needed for this phase of the work. For the first two years production of large quantities of the nanofibers and/or nanoparticles will occur followed-up by deployment on the vessel in the third year. Without this level of funding, only some of the conditions proposed could be investigated and the final system would not be ready for deployment on a fast response vessel. Delays only increase the risk that a chemical warfare

agent attack or other chemical contamination would have large negative impacts on the US population and the environment.

## **Basic Details**

### ***Description of Capability to be Delivered***

Upon completion of this project in six years, a set of nanoparticles and/or nanofibers that would be used for fast destruction and removal of a chemical contaminant from water will be ready for deployment on a fast response vessel.

### ***Target Acquisition Program***

The ability to treat and remove chemical contaminants from water is essential for a fast response vessel.

### **Approximate Program Timeline for Specific Capabilities**

- Year 1    The effect of the two nanoparticles on the three chemical contaminants will be identified. The effects of pH, salinity, UV exposure, temperature, and concentration (of contaminant and nanoparticle) will be identified. Optimized selection of nanoparticles completed.
- Year 2    Identification and quantification of byproducts formed during neutralization process completed.
- Year 3    Use of nanofibers as catalyst supports explored and optimized conditions for maximum destruction identified. The ability of nanofibers to act as adsorbents investigated and conditions for optimal destruction and removal of chemical contaminants found.
- Year 4    Scale-up conditions investigated. Sources for nanoparticle and/or nanofiber purchase identified or alternatives to synthesize in-house found. Purchasing/synthesizing initiated.
- Year 5    Completion of acquisition of required quantities of nanoparticles and/or nanofibers finished
- Year 6    Implementation of remediation capabilities on a response transport/vessel.

### **Technology Readiness Level (TRL) Estimate**

The Technology Readiness Level (TRL) of the current capability is between Level 2 and Level 3, as the effectiveness of the nanoparticles at the neutralization has been investigated; however, the use of nanofibers as adsorbents has not been tested in the laboratory and all the process variables have not been fully explored. The next step (for Level 3 compliance) is for the organization to undertake a characteristic proof of concept and an analysis to physically validate that the technology can be used, and can be demonstrated with innovative users.

## ***Strategy to Integrate to a Program***

### **Key participants**

Three PIs will participate on this project from Villanova University. Dr. Randy Weinstein of the Chemical Engineering Department will serve as lead PI. He will be in charge of the daily operations of the proposal and he has expertise in chemical catalysis and carbon nanofibers. Dr. Dorothy Skaf, also of the Chemical Engineering Department, will assist with the project as her expertise is in electrochemistry and UV catalyzed reactions. Finally, Dr. Amanda Grannas of the Chemistry Department will assist and bring her analytical and environmental chemistry knowledge to the project. We also expect to have 2 graduate students per year participate in the project as well as three undergraduate students each summer.

### **Anticipated contracting partners**

During the first three years only Villanova University participants will be involved with the project. Starting in year 4, outside sources for the desired nanoparticles and/or nanofibers will be identified. Finally, participants from Ablaze Development Corporation and their partners from the Navy will help with the integration of the final system onto the fast response vessel.

### **Expected funding levels**

Funding for the project is shown in the Appendix. The first three years of funding will go directly to Villanova University. The last three years of funding will be split between Villanova University (25%) and the outside company providing the nanofibers and nanoparticles (75%).

#### **Major Task Schedule and Funding (\$s in millions)**

Milestone Task	Required Funding (\$M)							
	FY 08	FY 09	FY 10	FY 11	FY 12	FY 13	FY 14	Total
Contract Approval								\$0.000
Development		\$0.250						\$0.250
Test & Evaluation			\$0.250	\$0.250				\$0.500
Production or purchase					\$1.000	\$1.000		\$2.000
Deployment							\$1.000	\$1.000
<b>Total</b>	\$0.000	\$0.250	\$0.250	\$0.250	\$1.000	\$1.000	\$1.000	\$3.750

### ***Capability Requirement Basis***

In the case of Sea Power 21, the Final Concept Report will be the primary source for the requirements along with the actual system to be placed on a vessel.

## Technical and Programmatic Details

### ***Current Status Summary***

Currently we have initial testing of the destruction of the three model contaminants in water with exposure to sunlight. For simplicity the results of the *m*-dinitrobenzene (DNB) neutralization will be discussed as similar procedures are used for each compound. For a standard test we first soak clear photo vials in 10% HCl solution for at least an hour to remove any residual contaminants from the glass surface. Next we place 0.01 g of nanoparticles ( $\text{TiO}_2$  or  $\text{ZnO}$ ) in each photovial and add 10 mL of 0.05 g/L solution of DNB in water (fresh or salt). Each photovial is covered with aluminum foil that has been cleaned with hexane to suppress any light catalyzed reaction until the start of an experiment. The photovial is capped and ready for use. For a typical experiment we place 32 photovials in the shaker under the UV lights in our sunlight simulator. After fixed intervals of time, photovials are removed and the contents transferred to amber vials and await analysis. Five mL of toluene is added to each amber vial to extract the DNB into the organic phase which is placed in a vortex to enhance contacting between the phases. The phases separate and are centrifuged, if needed to remove the nanoparticles before analysis. The toluene phase may be diluted to give a DNB concentration within the operating range of the electron capture detector (ECD) on the gas chromatograph (GC). Typical dilution is 5  $\mu\text{L}$  of toluene phase with 1 mL of toluene for injection into the GC.

Control experiments confirmed that over a 24 hour period DNB did not degrade in fresh or salt water even when exposed to simulated sunlight without the presence of nanoparticles. Without sunlight only minimal degradation occurs over 24 hrs (0-15%). Sunlight was required for any significant degradation and this can be accomplished naturally or with artificial UV light if sufficient sunlight is not available. We also tested that dilute hydrogen peroxide as well as purging all the oxygen out of the water with nitrogen had no effect on DNB degradation with  $\text{ZnO}$  particles. Hence the amount of dissolved oxygen in the water will not be critical for the destruction of DNB, extra oxygen (provided by hydrogen peroxide) or displacement of oxygen with nitrogen had no effect on DNB destruction. However,  $\text{TiO}_2$  particles required oxygen for the faster destruction rates and the removal of oxygen by nitrogen purging slowed down the reaction.

As a standard condition, we will compare results after 3 hours of sunlight and nanoparticle exposure. Our standard reaction conditions were 50 mg/L of DNB in water with a nanoparticle loading of 1 mg/mL. After 3 hours the  $\text{TiO}_2$  has complete destruction of the DNB while the  $\text{ZnO}$  had roughly 50% destruction. The addition of dirt to simulate turbid water at a high and low loading (8 mg/mL or 3 mg/mL) had detrimental effects to the destruction efficiencies. After 3 hours the results were somewhat scattered, but we never achieved more than 10-20% destruction when dirt was present in the water. The dirt was dry potting soil filtered through a Tyler mesh. However, if the dirt was dropped to only 1 mg/mL it had little effect on the  $\text{ZnO}$  destruction, but it did drastically slow down the destruction with  $\text{TiO}_2$ .

Our final initial test was to confirm that sea water was also a viable solvent for DNB destruction. We mixed our own solution of sea water from mineral salts to match those found in nature. We

found the  $\text{TiO}_2$  had slightly slower destruction rates while the  $\text{ZnO}$  had little change. From all of the results presented so far, fresh water, sea water, and dirty water, it appears that we can easily get DNB destruction by either  $\text{TiO}_2$  or  $\text{ZnO}$  nanoparticles in solution with the exposure to sunlight. No high powered UV sources were required. Similar results were found for the other model contaminants.

Although we have proven that the initial contaminate can be oxidized in an efficient manner, we have not investigated which, if any, byproducts are formed nor a method for fully oxidizing or removing these byproducts. In the next phase of the research we will employ carbon nanofibers as catalyst supports for the nanoparticles and as adsorbents to help remove materials from the water that are not fully oxidized. In the first stage of this research we will identify byproducts that are formed during the neutralization of each of the model compounds as a function of time, pH, salinity, and initial concentrations of the contaminants and nanoparticles. During the neutralization process we will also add carbon nanofibers and monitor their ability to remove contaminants and byproducts from solution by adsorption. Finally, we will support catalyst on the nanofibers to find a set of conditions that will increase the oxidation process and also effectively remove any contaminants and byproducts for solution. Our first approach for making nanoparticles on nanofibers will be with  $\text{TiO}_2$  through the hydrolysis and precipitation of titanium n-butoxide or  $\text{Ti}(\text{OBu})_4$ . In our first processes we will mix 20 mL titanium n-butoxide with 10 mL of anhydrous ethanol. This solution will then slowly be added at about 1 drop per 5 seconds to a 30 mL of a 50 % ethanol aqueous solution with vigorous stirring in which the carbon nanofibers are suspended. Upon contact with water, titanium n-butoxide instantly hydrolyzed to form  $\text{TiO}_2$  particles and released a vapor byproduct and heat. This addition will be done in an inert nitrogen atmosphere. SEM and TEM images of the dried nanofibers with catalyst particles will be obtained to know the coverage density and size of the particles. Stirring, concentration, and quantity of fibers used can be altered to improve the size and distribution of the nanoparticles on the fibers. Once formed, these nanofibers will be tested at how effectively they destroy the initial contaminants in aqueous solutions as was done previously with the nanoparticles alone.

## ***Risk Analysis***

There are no expected risks associated with this project. Final removal of nanoparticles and/or nanofibers can be accomplished by filtering and/or flocculation processes.

## ***Capability Development Strategy***

Current plans for research are described previously. In the second half of this project, large scale production or acquisition of nanoparticles and/or nanofibers will be accomplished. As we get closer to that time period, efforts will be made to identify potential sources or materials required or alternative methods for in-house production planned.

## ***Program Plan***

Year 1 Complete destruction of the three model contaminants obtained within 24hrs of exposure to nanoparticle/nanofiber combinations in both fresh and salt water in the laboratory (TRL 4).



- Year 2 Complete identification of all byproducts formed and ability to remove all material via oxidation and/or adsorption from solution within 24hrs of exposure (TRL 4).
- Year 3 Large scale test (100 gallons) for removal of contaminants and byproducts within 24hrs of exposure (TRL 5 and 6).
- Year 4 Confirming source of required nanoparticles/nanofibers for large scale production.
- Year 5 Obtain large scale quantities of nanofibers/nanoparticles and storage containers on fast response vessel.
- Year 6 Deployment on fast response transport/vessel.

*Note:* TRL beyond 6 is not feasible as it would require an actual chemical contaminant in a real waterway. Testing would only occur if a real accident or attack occurred.

## Chapter 3 - Microarray Identification of Pathogens

Villanova University

### Introduction

The overall goal of the initial study was to investigate the feasibility of establishing a fully-functional genomics facility within an oceangoing decontamination system that could be used for rapid detection of microbial pathogens including bioterrorism agents. For that purpose, two genotypic approaches for pathogen detection, namely polymerase chain reaction (PCR) based pathogen identification and microarrays, were evaluated between May 2007 and August 2008. The results suggest that PCR-based pathogen identification may not be a feasible technology for on-site use in an oceangoing vessel due to the fact that using this technology to screen for a large number of possible microbial pathogen might take a long time since each primer pair targeting a particular species has to be analyzed independent of the primer pair targeting another species. Microarrays, the so called gene chips, on the other hand were shown to be feasible for that purpose. During the feasibility assessment, the research team developed an effective protocol for extracting intact nucleic acids, both DNA and RNA, from highly complex matrices such as ocean water and biosolids, a step long considered to be the biggest challenge in the application of microarray technology for detecting pathogens in the environment.

This report provides a framework for further research and development of the concept that includes fabrication of microarrays, optimization, and development of Standard Operating Procedures. A tentative budget and schedule is included.

### Further research and development needs

The microarray technology is based on the principle of hybridization (hydrogen bonding) between complementary nucleotides that make up the genomic material, DNA or RNA. The complementary base pairing and other factors such as ionic strength, temperature, and solvents of the environment are the driving forces for hybridization. Regardless of the hybridization conditions, successful application of microarray technology dictates carefully chosen target genes. In other words, the gene or genes that will be targeted for a particular pathogen must be highly species-specific so that non-specific binding does not occur resulting in ambiguous identification. Thus, significant further research and development efforts must go into identification of target genes for each pathogen of interest. In addition, sufficient time must be spent in optimization of fluorescent labeling of nucleic acids (both DNA and RNA) extracted from ocean water samples and optimization of hybridization conditions.

### Basic Details

#### *Description of Capability to be Delivered*

The first step of using microarray technology is to extract intact (undigested) and contamination free nucleic acid from a sample. Then the nucleic acids are labeled with a fluorescent dye so that when the sample is hybridized with the microarray, the spots on the microarrays that the labeled

nucleic acids in the sample binds can be visualized using a fluorescent scanner. The effectiveness of the technology depends on the spots of microarray being complementary to the labeled nucleic acids.

Modern taxonomy is based on genotypic makeup of organisms. Specifically, the base sequence of genes in chromosomal DNA that encodes for 16S Ribosomal RNA (prokaryotes) and 18S (eukaryotes) is used to identify species and to determine relatedness of different species.

Developed by Carl Woese and George Fox (1977), RNA-based genotypic taxonomy and the new knowledge about the prokaryotic domain Archaea led to modern Three Domain classification of organisms (Madigan and Martinko, 2006). 16S and 18S ribosomal DNA (rDNA) sequences are highly conserved among a wide variety of organisms and therefore 16S and 18S rDNA based methods are extremely reliable and precise in identification of organisms (Bosshard et al., 2004).

A tentative list of waterborne pathogens and biological terrorism agents that are potential target organisms during a naval emergency is presented in the appendix. The first phase of Further Research and Development activities will be finalizing the list of target pathogens and developing a database of gene(s) that will be targeted for each of these pathogens. This will involve extensive literature review on each of these pathogens as well as combing two databases, GenBank and Ribosomal Database Project II, for species-specific gene markers. These are publicly available collections of known genes.

#### List of Potentially Target Pathogens

<b>PATHOGEN</b>	<b>DISEASE CAUSED</b>	<b>COMMON MODE OF TRANSMISSION</b>
<b>BACTERIAL PATHOGENS</b>		
<i>Bacillus anthracis</i>	Anthrax	Bioterrorism/airborne
<i>Yersinia pestis</i>	Plague	Bioterrorism/waterborne
<i>Francisella tularensis</i>	Tularemia	Bioterrorism/water-airborne
<i>Salmonella typhi</i>	Typhoid fever	Waterborne
<i>Salmonella paratyphi</i>	Paratyphoid fever	Waterborne
Shigella	Bacillary dysentery	Waterborne
<i>Vibrio cholerae</i>	Cholera	Waterborne
Pathogenic <i>E. coli</i>	Gastroenteritis	Water-foodborne
<i>Yersinia enterocolitica</i>	Gastroenteritis	Waterborne
<i>Campylobacter jejuni</i>	Gastroenteritis	Waterborne
<i>Legionella pneumophila</i>	Acute respiratory illness (legionellosis)	Airborne

<i>Mycobacterium tuberculosis</i>	Tuberculosis	Water-airborne
<b>VIRAL PATHOGENS</b>		
Polioviruses	Poliomyelitis	Waterborne
Coxsackieviruses A	Aseptic meningitis	Waterborne
Coxsackieviruses B	Aseptic meningitis	Waterborne
Echoviruses	Aseptic meningitis	Waterborne
Other enteroviruses	Encephalitis	Waterborne
Reoviruses	Mild upper respiratory and gastroenteritis	Waterborne
Rotaviruses	Gastroenteritis	Waterborne
Adenoviruses	Upper respiratory and gastrointestinal illness	Waterborne
Hepatitis A virus	Infectious hepatitis	Waterborne
Noraviruses	Gastroenteritis	Waterborne
<b>PROTOZOANS</b>		
<i>Acanthamoeba castellani</i>	Amoebic meningoencephalitis	Waterborne
<i>Balantidium coli</i>	Balantidosis (dysentery)	Waterborne
<i>Cryptosporidium</i>	Cryptosporidiosis	Waterborne
<i>Entamoeba histolytica</i>	Amoebic dysentery	Waterborne
<i>Giardia lamblia</i>	Giardiasis (gastroenteritis)	Waterborne
<i>Naegleria fowleri</i>	Primary amoebic meningoencephalitis	Waterborne
<b>ALGAL PATHOGENS</b>		
<i>Anabaena flos-aquae</i>	Gastroenteritis	Recreational water
<i>Microcystis aeruginosa</i>	Gastroenteritis	Recreational water
<i>Alphanizomenon flos-aquae</i>	Gastroenteritis	Recreational water
<i>Schizothrix caliciola</i>	Gastroenteritis	Recreational water

**Table 1: List of potentially target pathogens-Adopted from Pontius (1990) and expanded.**

Microarray fabrication, Phase II, will start once a list of target pathogens and species-specific gene database are compiled. It is important to note that two different types of microarrays might

have to be fabricated in order to screen for all of the pathogens of interest. This is simply due to the fact that certain pathogens, particularly viral pathogens, are detected using only DNA or RNA based microarrays since viruses carry only DNA or RNA, never both. Microarray fabrication will include the following steps.

1. Synthesis of species-specific gene markers, oligonucleotide probes, which will be used as the “spots” on the microarrays: Gene markers identified during Phase I for each of the target pathogen will be synthetically manufactured. This step will be subcontracted to a commercial facility that has the capability to synthesize oligonucleotide probes.
2. Fabrication of microarray(s): Microarray size and configuration, i.e. number of redundant probes for statistical purposes, will be based on the number of target pathogens and number of probes required for accurate identification of each target pathogen. Typical microarrays contain up to 96,000 gene probes on a single 75x25 mm slide. Assuming two probes will be sufficient for each target pathogen and four redundancies will be used, each microarray could target up to 12,000 pathogens. The microarray(s) design will be carried out at Villanova by the research team. However, fabrication will be subcontracted to a research or commercial facility. One possibility is to have the fabrication done at the Genomics and Microarray Facility of The Wistar Institute, University of Pennsylvania, where the PI was a visiting scholar during spring 2006. Villanova has had close collaborations with The Wistar Institute, which charges reduced internal rates for its services to Villanova.

Even when the target genes are chosen carefully, microarrays may produce ambiguous results unless hybridization conditions are optimized for the specific task the microarray is designed to accomplish. Accurate and effective hybridization between the microarray spots and labeled nucleic acids in samples is a function of particularly ionic strength of the hybridization solution and temperature used for hybridization. The research team will conduct a number of hybridization tests under different ionic strength and temperature conditions to determine the optimal hybridization conditions.

Successful implementation of any technology requires a well-trained work-force and clearly defined protocols that they can follow. During last phase of the proposed research, the research team will develop Standard Operating Procedures (SOP) that can be used by trained microbiologists working on an ocean-going vehicle. Separate SOPs will be developed for sample collection, extraction and purification of nucleic acids from those samples, labeling the extracted nucleic acids, hybridization of labeled nucleic acids with microarray(s), and finally interpretation of the results. Each SOP will include step by step instructions to carry out a particular task as well as Quality Assurance/Quality Control (QA/QC) required for each task.

### **Approximate Program Timeline for Specific Capabilities**

The proposed further research and development activities should take two (2) years from the start of the work. Below is a tentative schedule showing duration of each task explained in the previous sections.

Task \ Months	2	4	6	8	10	12	14	16	18	20	22	24
Project startup and training												
Phase I: Database development												
Phase II: Microarray fabrication												
Phase III: Optimization												
Phase IV: SOP development												

### ***Strategy to Integrate to a Program***

#### **Key participants**

It is expected that the key participants on this project will be from Villanova University. A break down of resources are included in the next section.

#### **Expected funding levels**

The estimated cost of further research and development efforts is \$314,186 for two years. The tentative cost of each line item is provided in Table 2. It is important to note that the proposed activities, particularly compiling the species-specific gene database require highly trained personnel and that is why a post-doctoral researcher must be included in the research team. In addition, the estimated cost includes Villanova University's indirect cost.

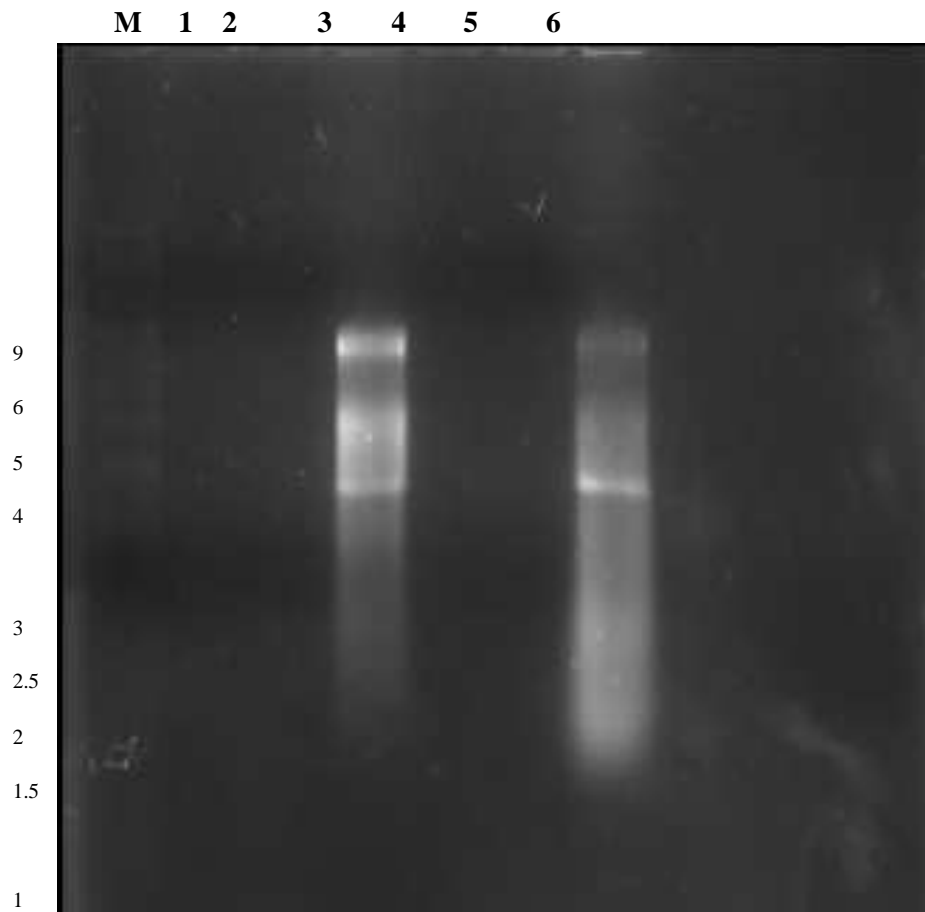
ITEM	COST
<b>Personnel</b>	
1 Post-doc (24 months)	90,000
1 Grad assistant (24 months)	36,000
Undergraduate students	15,000
PI (4 months)	36,000
<b>Personnel total</b>	<b>141,000</b>
VU Indirect cost (74.6% of personnel)	<b>105,186</b>
<b>Subcontracting</b>	

Probe synthesis	10,000
Microarray fabrication	20,000
<b>Subcontracting total</b>	<b>30,000</b>
Consumables for analytical testing	<b>30,000</b>
<b>Travel</b>	
Conferences	5,000
Workshops and other training	3,000
<b>Travel total</b>	<b>8,000</b>
<b>Project total (overall cost for 2 years)</b>	<b>314,186</b>

## Technical and Programmatic Details

### ***Current Status Summary***

Figure 1 depicts extracted nucleic acid from three different ocean water samples: blank (autoclaved ocean water sample without seeding); autoclaved ocean water seeded with *E. coli*; and autoclaved ocean water seeded with *Salmonella*. Lane M represent the RNA marker (standard) while Lane 1 through 3 represent the ethidium bromide stained nucleic acids extracted from the autoclaved ocean water samples with no seeding (blank), seeded with *E. coli*, and seeded with *Salmonella*, respectively. Lane 4 through 6 represent the same samples after DNAase treatment that removes any DNA extracted along with RNA.



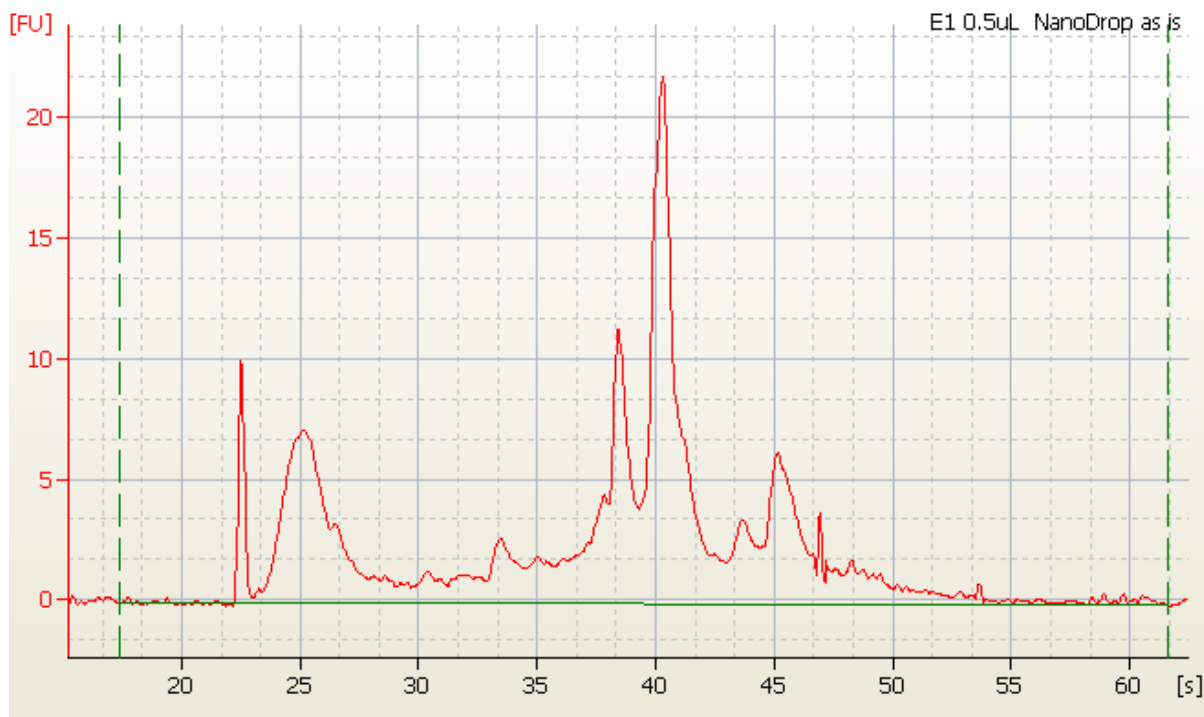
**Figure 1: Formaldehyde denaturing agarose gel electrophoresis of nucleic acids extracted from the seeded ocean water samples**

In order to verify that RNA was extracted, all of the three samples were treated with DNAase A (Sigma-Aldrich, Location) after the extraction. Lane 4 through 6 show the samples treated with DNAase. In other words, the samples shown in Lane 5 through 6 contain only RNA since any DNA in samples are degraded by actions of DNAase. Comparing samples in Lane 3 and 6, one clearly notices that the protocol is highly effective in extraction of clean (without DNA contamination) RNA from a complex matrix such as ocean water. Nucleic acid extracted from ocean water sample autoclaved and then seeded with *Salmonella* (Lane 3) basically showed no change after treatment with DNAase (Lane 6) indicating that the extraction solution was free from DNA, due most likely highly selective performance of the capture-column purification step. Two distinct bands in Lane 6, one about 3 kbp size and the other approximately 1 kbp, are likely to be 16S and 23S rRNA. Bands below 0.5 kbp are likely to be short messenger RNA pieces.

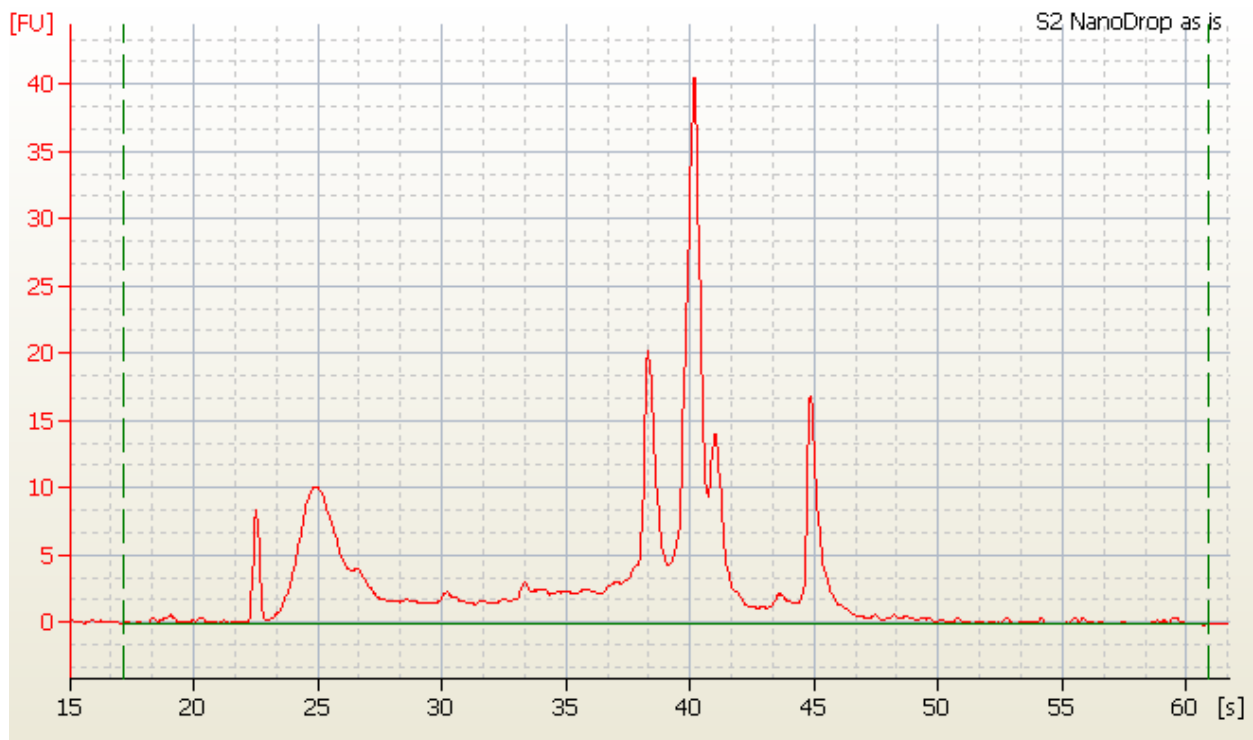
It should be noted that the results presented in Figure 1 are highly encouraging in terms of feasibility of using microarrays for pathogen identification. As mentioned earlier, extraction of sufficient clean and intact nucleic acid is the main challenge in applying the technology for pathogen detection and the research team has shown this challenge can be overcome. In order to verify that nucleic acids were extracted from the seeded ocean water samples, the quantity and



integrity of the RNA were also tested using an Agilent 2100 Bioanalyzer (Agilent, Wilmington, DE), standard RNA integrity analysis. The bioanalyzer analyses were conducted at the Core Genomics Facility of the Wistar Institute, Philadelphia, PA. Figure 2 and 3 show the electropherograms of RNA extracted from seeded ocean water sample.



**Figure 2: Agilent Bioanalyzer electropherogram of RNA extracted from ocean water spiked with *E. coli*.**



**Figure 3: Agilent Bioanalyzer electropherogram of RNA extracted from ocean water spiked with *Salmonella* sp.**

The significance of bioanalyzer results lays in bands labeled as 23S rRNA in Figure 2 and 3. The 23S rRNA peaks are the indicators of RNA degradation since 23S RNA is the first form of RNA that degrades during extraction or preservation of extracted samples. Shorter 23S rRNA peak in Figure 2, which shows RNA extracted from ocean water sample seeded with *E. coli*, and following jagged signal indicate that RNA is at least partially degraded. Although it is still possible to achieve good microbial identification from 16S RNA in that sample, partially degraded RNA may not suitable for certain applications such as gene expression analysis. However, the sharp shape of the 23S RNA peak in Figure 3 and the smooth signal following it indicate that the RNA extracted from the other ocean sample seeded with *Salmonella* sp. is in perfectly intact condition, free of any degradation. Figure 3 strongly suggest that the extraction protocol developed during this study can successfully be used to obtain sufficient quantities of intact RNA from seeded ocean water samples. It is important to note that partial degradation of RNA extracted from the ocean water sample seeded with *E. coli* might have happened during transportation of the extracted RNA from Villanova to Philadelphia and it may not be an issue if samples could be analyzed on site immediately after extraction.

The results presented in this section strongly suggest that extraction of sufficient quantities of intact nucleic acid, both RNA and DNA, from a complex matrix such as ocean water is possible using commercially available extractions kits and optimized extraction protocols. This is a

significant development since application of any genotypic method to microbial identification in ocean water samples will require sufficient quantities of intact nucleic acid. The research team made significant progress towards overcoming this major challenge as evidenced by the results presented in this report. These findings indicate that a microarray-based pathogen identification approach is feasible on an oceangoing emergency decontamination vehicle.

### ***Program Plan***

Year 1    Developing target pathogen list and species-specific gene database.

Year 2    Fabrication of microarray(s); development of Standard Operating Procedures.

### **References**

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## ***Chapter 4 – Enhanced Resolution Nuclear Radiation Detector***

Villanova University

### **Introduction**

#### ***Purpose***

We propose a compact enhanced resolution nuclear radiation detector based on quantum dot (QD) and microstructured optical fiber (MOF) technologies. The preliminary version of the detectors will sense gamma-rays that are common radiation artifacts of weapons grade nuclear materials. The next generation of detectors will detect neutrons and alpha particles. The proposed techniques allow for the composition of an inorganic semiconductor/glass detection system that is nuclear radiation resistant and addresses the issue of signal degradation in glass by using an air-core light guide coated with QDs to transmit the scintillation signal to electronic processing equipment. The transmission length of the QD coated optical fiber is also increased to improve scintillation detection and to reduce the number of electronic signal processing stations.

The scintillation of the QDs under gamma radiation demonstrate favorable energy resolutions to that of standard sodium iodide NaI(Tl) scintillators.<sup>1</sup> There are disadvantages to using conventional gamma radiation detection techniques such as bulk semiconductors (e.g. germanium) and glass scintillators (e.g. NaI(Tl)). Germanium, which although provides good resolution, is limited in operation due to temperature dependencies that confines operation conditions to liquid nitrogen temperatures. The conventional scintillator, sodium iodide with thallium (NaI(Tl)), is not restricted to prescribed operation temperatures but suffers from poor energy resolution which is approximately 7% energy resolution at 662 keV.<sup>1</sup>

The main advantage of the reduced size of QDs is the increase in the material band gap energies which promotes the efficient emission of photons in the visible region.<sup>2</sup> This visible luminescence feature can be used for scintillation purposes. Efficient photon counting and high photon output are essential for photon detection of scintillation light. The QD coating process can be achieved using a recently developed pressure driven approach making mass production of these QD filled fibers reasonable.<sup>3</sup>

Detection efficiency and energy resolution are key principles in gamma-ray radiation detection.<sup>4</sup> QD gamma-ray detection efficiency is typically low due to its low QD density and low average atomic number  $Z$ . The interaction length between the gamma rays and QD materials may be increased via coating the hollow core surface with the QDs. The total count of gamma-ray quanta that interact with the detector may be enhanced. Additionally, the QD material can offer enhanced photon generation in the visible region for improved compatibility with existing scintillation collection schemes (e.g. photomultiplier tubes). For example, it has been reported that CdSe/ZnS QDs that luminescence at 510nm when exposed to gamma ray energies of 59 keV generate more visible photons than a conventional NaI detector.<sup>1</sup> So, although the signal-to-noise ratio for the NaI crystal is attractive due to its higher detection efficiency (via its density and size), the energy

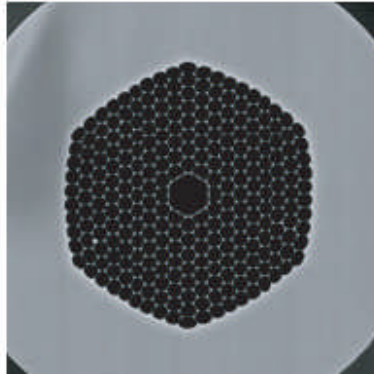
resolution can be improved for composite QD materials due to the enhanced scintillation qualities.

## **Summary**

Nuclear particle detection and identification are of growing importance to the military and to society as a whole. Threats of nuclear attacks or accidents exist<sup>5</sup> that have the potential to affect deployed vessels and to affect civilian populations at nearby ports. A compact, light weight, reliable optical fiber based nuclear radiation contamination detector would be beneficial to a seagoing decontamination system. A QD coated glass optical fiber scintillation detectors for sensing gamma particles are proposed. The detectors presented offer advantages such as ruggedness since they are not susceptible to nuclear radiation degradation, humidity, and drastic temperature variations which in addition to portability make the fiber nuclear detection system a good candidate for oceangoing vessels. Oceangoing vessels can be subjected to weather conditions of severely cold or hot climates with precipitation and it is important to have instrumentation, especially for ships with radioactive cargo that have reliable nuclear detection, without sacrificing cargo space. The sensor components are also immune to electromagnetic interference from electromagnetic pulses, EMPs, which may accompany nuclear attacks or accidental explosions. The results obtained from these experiments may also demonstrate the potential usage of QD-MOF detectors in alpha, neutron and gamma-ray detection which can be used in medical imaging, environmental monitoring as well as security and defense.<sup>1,6,7</sup>

The QD coating process of the MOF can be achieved using a recently developed pressure-driven MOF filling approach making mass production of these QD filled fibers reasonable.<sup>8</sup> Soluble forms of QDs can be used in the aqueous process of layer by layer electrostatic self assembly (ESA) deposition technique.<sup>9</sup> ESA technique allows precise control over the final properties of the film, material selection and deposition parameters. In comparison to other deposition techniques, such as sol-gel, it offers the benefit of control on the nanometer scale of the thickness of the coating, and the possibility of using different combinations of anionic and cationic colloids for the fabrication of the coatings.

The proposed detectors will incorporate a microstructured optical fiber to transmit the scintillated light from the QD thin film coating in the core region due to radiation exposure. Microstructured optical fibers (MOFs) are specialty optical fibers in which a series of carefully spaced periodic micron-sized cavities within an air-silica lattice in the cladding of the fiber provide extraordinary waveguide characteristics not demonstrated by standard optical fibers. (See Fig.1.) One advantage of this approach is to possibly minimize the radiation absorption losses that can attenuate and distort the scintillation light by using air-core MOFs. Air-core MOFs demonstrate a modified photonic band gap confinement such that the fiber can be designed to support the propagation of light of a desired spectral range along the air-core.<sup>10,11</sup> This unique air guidance property may permit scintillation light to propagate through the air-core thereby avoiding the absorption that occurs at scintillation wavelengths in the solid-core region of a conventional optical fiber. This system may also offer the possibility of longer transmission fiber lengths such that the electronic signal processing equipment location can be removed from the radiation site, avoiding both radiation and EM interference.



**Figure 4: Cross-section of a Corning Photonic Band Gap™ specialty fiber. Typical core diameters range from 8μm to 12μm. The fibers are standardized to have a 125-150μm cladding diameter.**

## Basic Details

### ***Description of Capability to be Delivered***

#### **Task 1. Characterize the QD thin film MOF.**

Techniques for coating the hollow core fiber with the QD thin film will be established. Optical measurements will involve transmission attenuation for a range of scintillation wavelengths and lengths of QD-MOF using Hamamatsu™ photomultiplier tubes with an Ortec digiBASE™ system and an Ando™ optical spectrum analyzer. This characteristic is important to the determination of optimal QD materials and thin film thicknesses to maximize detector efficiency.

#### **Task 2. Characterize the QD-MOF detector**

The long interaction length of fibers may lead to significant detector efficiency enhancement as well as improved energy resolutions.

#### **Task 3. Characterize QD-MOF at various scintillation wavelengths**

The scintillation wavelength will be identified for the QD scintillation materials that experience the highest luminescence for the scintillation process. This information will also determine the appropriate settings for the PMT and digiBASE signal processing electronics. The characterization process will measure the detector efficiency as a function of optical fiber amplifier and MOF length.

#### **Task 4. Determine the detector response as a function of environmental conditions**

Detector will be characterized to determine detector efficiency as a function of temperature, humidity, vibration and background radiation. These measurements will determine the reliability of the detector in environmental conditions that are common to oceangoing vessels. These sensors may have the potential to withstand fires and temperature variations that often accompany a nuclear incident.

#### **Task 5. Determine the lifetime of the detector**

Measurements will determine the amount of radiation damage the inorganic QD-glass fiber detection system will sustain before failure of the detector. Detection efficiency curves will be composed as a function of scintillation wavelength and comparisons will be made between results

taken before and after extended exposure times and high radiation doses. This information will identify the lifecycle of these detectors for maintenance (i.e. annealing to reverse radiation damage) or replacement.

**Task 6. Proof-of concept sensor /final report**

Detection system will be assembled in a compact and efficient arrangement. The detector will be packaged in a hermetically sealed container to promote ruggedness and system portability.

***Target Acquisition Program***

Common weapons grade material used in warfare technology and possible cargo for Navy vessels can be composed of uranium radioactive material. Gamma-ray detection is important in the detection of weapons grade uranium.<sup>12</sup> The goal of this proposed work is to develop a compact enhanced-resolution gamma-ray nuclear detector that is resistant to environmental interference. The detector will use a QD thin film scintillator to coat an all glass optical fiber system that may provide radiation resistance to the detection system. The optical fiber amplifier will compensate for the short attenuation lengths normally associated with glass. A number of patents exist on optical amplification of nuclear radiation detection.<sup>13,14</sup> However, the scenario of a QD-MOF based optical fiber scintillation system has not been presented.

**Include dates for specific capabilities**

<u>Year 3</u>	QD detector efficiency curve with and without MOF. Detector efficiency curve as a function of QD-MOF length.
<u>Year 5</u>	Detector efficiency curve as a function of proximity to the radioactive source. Detector efficiency curve as a function of scintillation wavelength via different QD materials
<u>Year 6</u>	Detector efficiency curve as a function of temperature, humidity, vibration and background radiation.
<u>Year 7</u>	Detector efficiency curve before and after extended exposure to radiation as a function of scintillation wavelength.

**Provide an estimate of the Technology Readiness Level (TRL)**

The Technology Readiness Level (TRL) of the current project is between Level 2 and Level 3, as indicated. Speculative applications have been proposed on paper and in theory although not in practice. There is no proof or detailed analysis to support the overall assumptions. The next step (for Level 3 compliance) is for the characteristic proof of concept and an analysis to physically validate that the technology can be used, and can be demonstrated.

## Strategy to Integrate to a Program

### Key participants

The project milestones have been presented to determine the detector characteristics. The tasks will be performed at Villanova University by Dr. Wynne along with her graduate and undergraduate research assistants.

Direct labor: The PI will dedicate 1 month to the project and will charge 1 month's summer salary to the project (over the fiscal year) per fiscal year during the proposed 6 year period. There will be 1 full time graduate student salary supported on this project in addition to 2 undergraduate research assistants (per fiscal year). The graduate student will be doing full time research on this project. Tuition will be covered by Villanova University if full overhead is supported by grant.

Indirect Costs: Include 75.2% of direct labor costs. Please refer to the attached Colleges and Universities Rate Agreement document.

Materials: Material costs include chemicals and consumables to manufacture and study the detector. All of the tasks including detector characterization and the fabrication of a "proof-of-concept" detector will be the tasks accomplished with the materials money.

### Expected funding levels

Major Task Schedule and Funding (\$s in millions)

Milestone Task	Required Funding (\$M)								Total
	FY 08	FY 09	FY 10	FY 11	FY 12	FY 13	FY 14		
Contract Approval	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	
Development	\$ -	\$ 0.10	\$ 0.10	\$ 0.05	\$ 0.05	\$ 0.05	\$ 0.05	\$ 0.40	
Test & Evaluation	\$ -	\$ 0.05	\$ 0.05	\$ 0.10	\$ 0.10	\$ 0.10	\$ 0.10	\$ 0.50	
Certification Award	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	
S T E	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	
1 <sup>st</sup> Procurement	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	
1 <sup>st</sup> Deployment	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	\$ -	
Total	\$ -	\$ 0.15	\$ 0.15	\$ 0.15	\$ 0.15	\$ 0.15	\$ 0.15	\$ 0.90	

### Fiscal Year Break Down

ITEM	COST
Direct Labor	\$19,440
Indirect Costs	\$14,619
Publications	\$2,000
Materials	\$96,000
Other Direct Costs	\$9,941
Travel	\$8,000
<b>TOTAL</b>	<b>\$150,000.00</b>

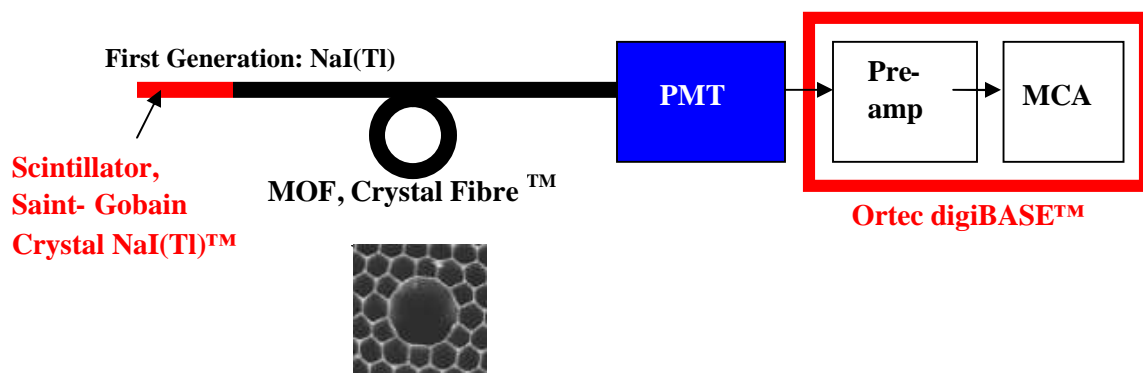


## Technical and Programmatic Details

### ***Current Status Summary***

#### Design for Scintillation Detection System

The design of a customized system for the gamma ray scintillator based on NaI(Tl) and MOF is finalized and novel compact QD-MOF scintillation detector with enhanced resolution is proposed. The previous first generation system consists of a NaI(Tl) scintillator that is coupled to a microstructured optical fiber. The scintillated light (at  $\lambda=415\text{nm}$ ) will be transmitted through the fiber to be amplified by a photomultiplier and then undergo signal processing. The second generation system will consist of a QD-MOF scintillator that will replace the NaI(Tl) glass scintillator (See Fig.2.) The effective attenuation that the photon experiences as it propagates along the fiber system is dependent on the scintillation wavelength as a result of the fiber material properties. A reduced effective attenuation can be achieved by transmitting the photon via an air transmission region such as the hollow core of the fiber that may lead to an exponential increase of photons surviving the fiber transmission process.<sup>15</sup> One advantage of this approach is to possibly minimize the radiation absorption losses that can attenuate and distort the scintillation light by using air-core MOFs. Air-core MOFs demonstrate a modified photonic band gap confinement such that the fiber can be designed to support the propagation of light of a desired spectral range along the air-core.<sup>16,17</sup> (See Fig.3.) This unique air guidance property may permit scintillation light to propagate through the air-core thereby avoiding the absorption that occurs at scintillation wavelengths in the solid-core region of a conventional optical fiber. This system may also offer the possibility of longer transmission fiber lengths such that the electronic signal processing equipment location can be remote from the radiation site avoiding both radiation and EM interference.



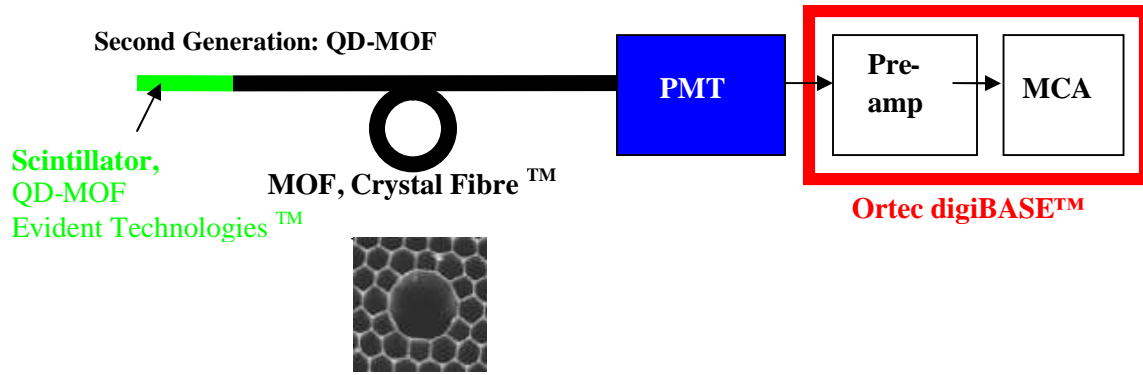


Figure 5: The gamma radiation detector concept: first and second generation.

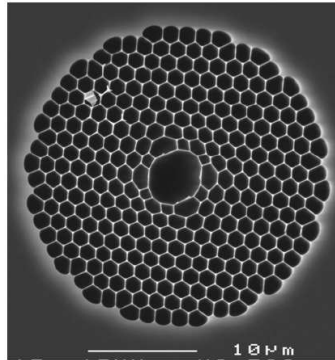
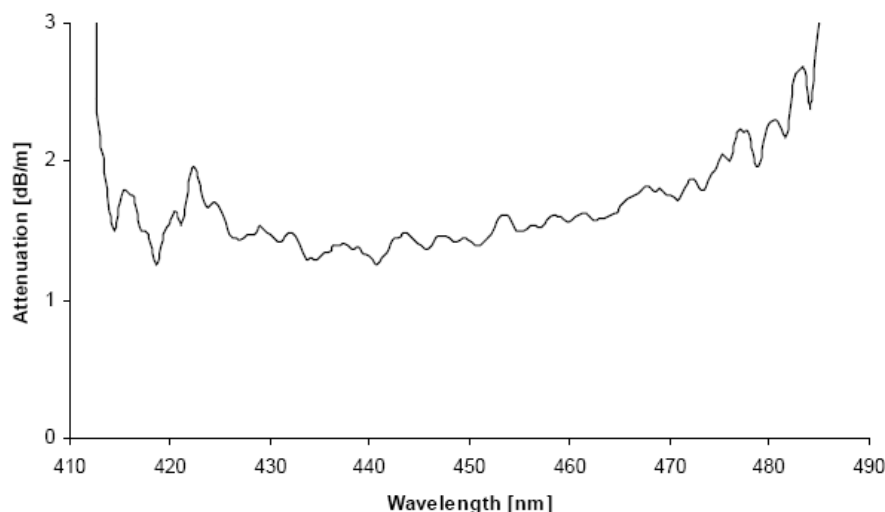


Figure 6: Cross-section of a Crystal Fibre™ HC-440-01 'blue' hollow core photonic bandgap fiber.<sup>18</sup> The core diameter 4.9μm. The fibers has an 84μm cladding diameter.

The microstructured optical fiber has a transmission window such that the scintillated light could propagate through the air core with relatively low loss and low bend sensitivity. (See Fig.4.) The probability<sup>15</sup>,  $P(x)$ , that a photon travels some distance  $x$  along the optical fiber away from its position of origin in the scintillator is

$$P(x) = A \exp(-\mu x)$$

where  $A$  is a constant that is a function of the geometry of the scintillator,  $\mu$  is the effective attenuation the photon experiences as it propagates along the fiber system. The attenuation is dependent on the scintillation wavelength which is due to the fiber material properties. If the effective attenuation can be reduced by transmitting the photon via a low index or air transmission region, the likelihood of photons surviving the fiber transmission process may increase exponentially.



**Figure 7: Typical attenuation for MOF Crystal Fibre™ HC-440-1<sup>19</sup>**

Gamma rays are typically detected with scintillation detectors. The gamma radiation is converted into a light pulse. Efficient gamma ray scintillation detectors are large in size and are very dense. Thallium activated sodium iodide (NaI(Tl)) crystals are commonly used for gamma radiation detection. NaI(Tl) has a peak emission wavelength that is closely matched to the bialkali PMT sensitivity curve (See Fig. 5.) such that it produces a stronger signal than most other scintillation materials for an amount of energy absorbed. The preliminary system will be designed to detect gamma radiation from a <sup>137</sup>Cs sample. As <sup>137</sup>Cs decays it emits gamma radiation with energy 662 keV in addition to X-ray and beta ray energies. (See Fig.6.)

Detector efficiency and energy resolution are key principles in gamma-ray radiation detection.<sup>20</sup> The detection efficiency (or stopping power) is a function of the detector material density and dimension. Energy resolution is influenced by the counting statistics of the particles generated during the gamma ray interaction with detection material. QD gamma-ray detection efficiency is typically low due to its low QD density and low average atomic number Z. The interaction length between the gamma rays and QD materials may be increased via coating the hollow core surface with the QDs. The total count of gamma-ray quanta that interact with the detector may be enhanced. Additionally, the QD material can offer enhanced photon generation in the visible region for improved compatibility with existing scintillation collection schemes (e.g. photomultiplier tubes). For example, it has been reported that CdSe/ZnS QDs that luminescence at 510nm when exposed to gamma ray energies of 59 keV generate more visible photons than a conventional NaI detector.<sup>1</sup> So, although the signal-to-noise ratio for the NaI crystal is attractive due to its higher detection efficiency (via its density and size), the energy resolution can be improved for composite QD materials due to the enhanced scintillation qualities.

From the measured energy resolution,  $\Delta E/E$ , the number of photons generated in the material under gamma ray exposure at a given energy can be determined to accurately identify radiation sources. For an ideal scintillator the energy resolution<sup>1,4</sup> R is given by

$$R = (\Delta E/E) = \sqrt{\frac{1 + v(M)}{N \cdot p}}$$

N is the average number of photons generated at a given energy E,  $v(M)$  is the variance of multiplication factor of the PMT (for a typical PMT with a gain of  $2.10^6$ ,  $v(M)$  is approximately 0.08) and p is the average transport efficiency.

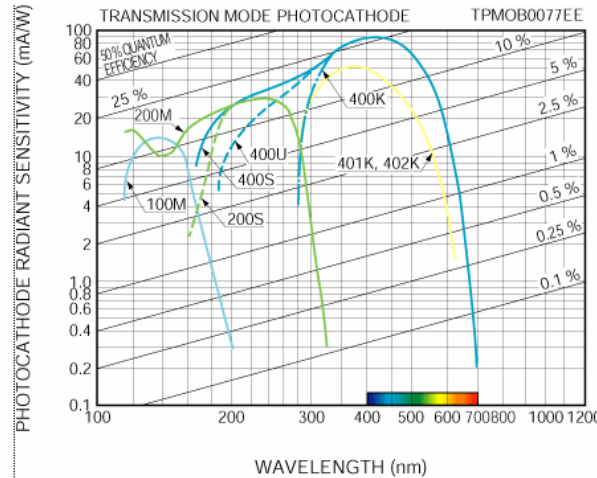


Figure 8: PMT spectral response of a Hamamatsu™ R1635 PMT (spectral response curve 400K)<sup>21</sup>

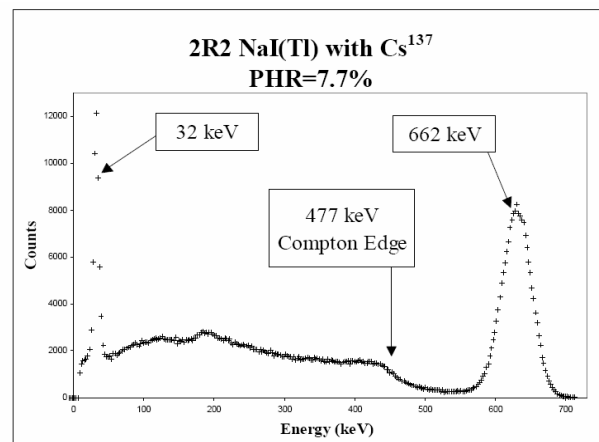
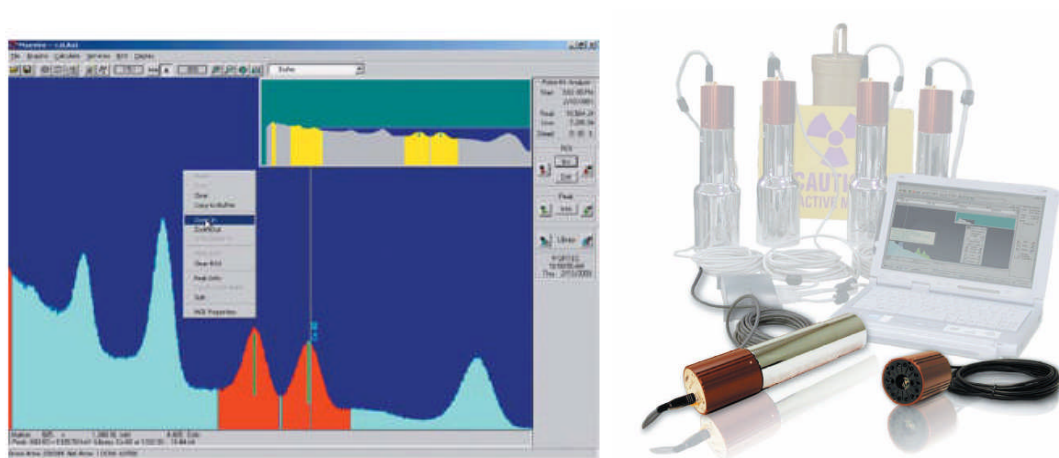


Figure 9: <sup>137</sup>Cs radioactive source measured with a 2"x2" NaI(Tl) detector.<sup>22</sup>

### Basic Scintillation Scheme

The scintillator will be a QD material (e.g. CdSe/ZnS core-shell) coated length of hollow core fiber (Crystal Fibre HC-440-1™) with low transmission losses for the scintillation light transmitted to the photodetector. The low atomic number of the CdSe/ZnS dots results in a low stopping power. In order to detect high-energy gamma rays radiation sources for contamination remediation efforts, dots with a higher atomic number, such as PbS are recommended.<sup>1</sup> The PMT

(Hamamatsu-Photonics R1635) will produce an amplified electrical current in response to the scintillation signal. The electrical signal is preamplified (Ortec digiBASE™) and sent to be analyzed with a multichannel analyzer (MCA). The system also has a graphic user interface for qualitative and quantitative spectrum analysis. (See Fig.7.)



**Figure 10: Typical Digibase spectral analysis and apparatus.<sup>23</sup>**

#### Evaluation:

The techniques discussed earlier allow for the composition of an inorganic QD-glass fiber detection system that is nuclear radiation resistant and addresses the issue of signal degradation in glass by using an air-core light guide to transmit the scintillation signal to electronic processing equipment. The detectors presented offer advantages such as ruggedness since they are not susceptible to nuclear radiation degradation, humidity, and drastic temperature variations which in addition to portability make the all glass fiber nuclear detection system a good candidate for oceangoing vessels. Oceangoing vessels can be subjected to weather conditions of severely cold or hot climates with precipitation and it is important to have instrumentation especially for ships with radioactive cargo that have reliable nuclear detection without sacrificing cargo space. The detector offers added benefits such as being chemically non-corrosive and having the potential to be “connectorized” to allow for low-technical-level-skilled personnel to install and operate the system.

#### Conclusion:

A compact, light weight, reliable optical fiber based nuclear radiation contamination detector would be beneficial to a seagoing decontamination system. A compact QD-MOF nuclear radiation detector based on nano-semiconductor and microstructured optical fiber technology will be developed. The detectors will sense neutrons and gamma-rays that are common radiation artifacts of weapons grade nuclear materials. Packaged detectors can be located aboard military vessels to monitor nuclear reactors that power ships and submarines, nuclear cargo, and nuclear warfare technology.

## ***Risk Analysis***

As explained in the previous sections present conventional gamma ray detection capabilities suffer from low resolution (e.g. sodium iodide crystal) and limited operating temperatures (e.g. germanium detectors). A new class of detectors are being developed that consists of nano-composite materials that have scintillation properties that can be controlled via the assembling of nanometer sized semiconductor crystals. The important properties of QDs such as quantum efficiency and emission wavelength can be tuned by changing the geometry, composition and size of these components. A standard NaI(Tl) crystal emits scintillation at a wavelength of 460 nm. But the present photomultiplier tube (PMT) has a maximum efficiency of 25% at these wavelengths. It is possible to tune the output wavelengths when QDs are employed as the scintillating medium matching photodiode specifications with quantum efficiencies as high as 70%.<sup>6</sup>

Purely inorganic QDs solids may not be the optimal candidate for the QD-MOF detection system they may exhibit some charge carrier trapping and absorption<sup>1</sup> at scintillation wavelengths resulting in lost output. A viable solution to these limitations is the composite QD scintillator, which are comprised of an inorganic semiconductor QD and organic semiconductors. These composite QDs have the cost and processing advantages of organic scintillators and the ionization characteristics of inorganic semiconductors.<sup>7</sup>

## ***Program Plan***

- Year 2      Characterize the QD materials
- Year 3      Characterize the QD-MOF
- Year 4      Characterize QD optical fiber detector efficiency at scintillation wavelengths
- Year 5      Determine the sensor response as a function of environmental conditions
- Year 6      Determine the lifetime of the detector
- Year 7      Proof-of concept sensor /final report

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## Appendix A: Technology Readiness Level (TRL)

Level	Technology Readiness Level	Description	Commonly used terms from the Hardware / Systems perspective	Practice-Based Technologies (PBTs), a proposed alternative explanation
1	Basic principles observed and reported.	Lowest level of technology readiness. Scientific research begins to be translated into applied research and development. Examples might include paper studies of a technology's basic properties.	Scientific research, paper studies	Scientific, behavioral, and market research, paper studies
2	Technology concept and/or application formulated.	Invention begins. Once basic principles are observed, practical applications can be invented. The application is speculative and there is no proof or detailed analysis to support the assumption. Examples are still limited to paper studies.	Practical, speculative applications invented	Practical, speculative applications invented, potential user communities identified
3	Analytical and experimental critical function and/or characteristic proof of concept.	Active research and development is initiated. This includes analytical studies and laboratory studies to physically validate analytical predictions of separate elements of the technology. Examples include components that are not yet integrated or representative.	Active R&D initiated, analytical and lab studies of components	Active R&D initiated, critical elements identified and demonstrated with innovative users
4	Component and/or breadboard validation in laboratory environment.	Basic technological components are integrated to establish that the pieces will work together. This is relatively "low fidelity" compared to the eventual system. Examples include integration of "ad hoc" hardware in a laboratory.	Basic components integrated, lab environment	Basic elements integrated to form core PBT, visionary leaders used to demonstrate value and transitionability
5	Component and/or breadboard validation in relevant environment.	Fidelity of breadboard technology increases significantly. The basic technological components are integrated with reasonable realistic supporting elements so that the technology can be tested in a simulated environment. Examples include "high fidelity" laboratory integration of components.	Integrated components demonstrated in simulated environment	Prototypes of implementation mechanisms established, demonstrated with core PBT for pragmatic users in simulated environments, such as role-based workshops
6	System/subsystem model or prototype demonstration in a relevant environment.	Representative model or prototype system, which is well beyond the breadboard tested for TRL 5, is tested in a relevant environment. Represents a major step up in a technology's demonstrated readiness. Examples include testing a prototype in a high fidelity laboratory environment or in simulated operational environment.	Prototype tested in relevant environment	Implementation mechanisms refined and integrated with core PBT, demonstrated in relevant environments, e.g., pilot settings
7	System prototype demonstration in an operational environment.	Prototype near or at planned operational system. Represents a major step up from TRL 6, requiring the demonstration of an actual system prototype in an operational environment, such as in an aircraft, vehicle, or space. Examples include testing the prototype in a test bed aircraft.	Actual system prototype in operational environment	Implementation needs of mainstream users identified and integrated into the prototype, operational use by relevant users demonstrated across the community
8	Actual system completed and qualified through test and demonstration.	Technology has been proven to work in its final form and under expected conditions. In almost all cases, this TRL represents the end of true system development. Examples include developmental test and evaluation of the system in its intended weapon system to determine if it meets design specifications.	Final form proven to work in operational environment	Technology picked-up for wide-spread rollout across the community

9	Actual system proven through successful mission operations.	Actual application of the technology in its final form and under mission conditions, such as those encountered in operational test and evaluation. In almost all cases, this is the end of the last “bug fixing” aspects of true system development. Examples include using the system under operational mission conditions.	Actual application running under mission conditions	PBT use is considered routine within community, best practices and body of knowledge in place
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